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Breast Cancer

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13. ABSTRACT ( <i>Maximum 200 Words</i> )  The goal of the studies is to determine optimal strategies for combining radiation and drug therapies that destroy tumor vasculature using murine tumors and to elucidate microenvironmental (blood flow, oxygen, etc.) changes in the tumor to guide the planning of combined treatment. The specific aims are:  <u>Aim 1:</u> To measure the response of murine tumors to combined radiation and drug therapies that target tumor vasculature under variations of order and timing of the two modalities. <u>Aim 2:</u> To measure microenvironmental changes such as blood flow and tumor oxygen following the combined therapies.  The results to date have resulted in one published paper, two papers in preparation and three scientific presentations. The significant events to date include:  1. Blood flow changes are predictive of tumor response following drug therapy that is designed to target tumor vasculature. 2. Radiation therapy sensitizes drug therapy with respect to tumor response in either a single dose regimen or a fractionated schema. 3. Timing of radiation and drug therapy is critical to the magnitude of tumor response. If radiation follows drug administration, the effect of the therapies on tumor response is additive. If radiation proceeds drug delivery by 1 hour, the effect of the therapies on tumor response is more than additive. 4. The magnitude of blood flow decrease 60 hours following the combined treatment of radiation and drug is consistent with and predicts increased tumor response.			
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NMR with Combined Antiangiogenic and Radiation Therapies - Breast Cancer

Introduction

New therapies that target tumor vasculature are receiving intense attention because they exhibit their effect preferentially on tumor vasculature compared with that of normal tissue. New therapies are combined with conventional treatment; radiation therapy is a standard therapy for breast cancer. There are reasons to believe timing of drug and radiation would be important. The proposal is designed to investigate the effect of sequence and timing on tumor response following the combined administration of drug and radiation. Furthermore, non-invasive tools for measuring blood flow and oxygen are used which have a potential direct clinical utility.

NMR with Combined Antiangiogenic and Radiation Therapies - Breast Cancer

Body – Annual Progress Report

The research accomplishments in the last year have been significant. We have completed studying the tumor response to two drug therapies that target tumor vasculature alone and in combination with radiation therapy. The two drug therapies used were combretastatin (kindly provided by OxiGene, Sweden) and arsenic trioxide (purchased from Sigma Scientific). Our results indicate that in response to drug alone, there is a small but significant tumor response. In combination with radiation, antivascular therapies are much more effective especially in a fractionated regimen (Lew et al. 1999 for arsenic trioxide; Brown et al. 2001 for combretastatin). This represents a completion of Task 1 (using two separate antivascular therapies).

We have also measured changes in tumor physiology (specifically, blood flow and oxygen) in response to the drug therapies with and without radiation. We have completed the tumor blood flow measurements (using  $^{86}\text{Rb}$  uptake and  $^{14}\text{C}$ -iodoantipyrine quantitative autoradiography, QAR). Of note is the completion of the QAR blood flow measurements using spontaneous breast carcinoma, since it was a major technical accomplishment to perform QAR in mice (the challenge results from a small mouse blood volume necessarily sampled over time to obtain a quantitative blood flow determination). We have completed our measurements of tumor oxygen in response to arsenic trioxide (using Eppendorf glass electrodes in collaboration with Dr. C.W. Song, University of Minnesota). These studies are encompassed by Task 2. A major component of Task 2 is to extend the invasive measurements of tumor oxygen and blood flow to non-invasive MRI measurements of tumor oxygen and blood flow. To date, MRI measurements of blood flow and oxygen remain a challenge.

NMR blood flow and  $^{19}\text{F}$  oxygen studies are progressing slower than expected due to technical difficulties. The implementation of the MRI three-dimensional blood flow measurement called FAIR has been problematic. However, in collaboration with our consultant, Ralph Mason, University of Dallas Southwestern Medical Center,  $^{19}\text{F}$  oxygen studies in tumors have been initiated. The major focus of our efforts in the third year of the grant is to complete Task 2. We anticipate this to be feasible. In summary, the project is progressing as planned with the tumor response studies ahead of schedule and the tumor physiology measurements behind schedule.

Some significant findings have resulted from our invasive measurements of tumor physiology. We found a positive correlation between blood flow decrease in response to drug therapies that target tumor vasculature and tumor response (Illustrated in Figures 1 and 2 for combretastatin). Furthermore, under optimal conditions of sequence and timing, radiation therapy combined with drug therapy enhances both the tumor blood flow reduction (see Figure 3 for blood flow changes to arsenic trioxide) and eventual tumor response (as measured by growth delay, see Lew et al. 2000). The suggestion is that

radiotherapy acts to sensitize cancer to drug therapies that target tumor vasculature. Our measurements of tumor oxygen to date suggest that antivascular agents actually improve tumor oxygen (Lew et al. 2000), contrary to conventional wisdom. One hypothesis is that arsenic trioxide causes cellular death within the tumor leading to decreased oxygen consumption and increased oxygen availability. Our observation of tumor response to fractionated radiation plus drug regimen is consistent with this theory (Lew et al. 2000 and Figure 2). Finally, our results indicate that blood flow changes in response to the combined treatment of radiation and drug therapies can be used to predict tumor response to the combined treatment.

The tasks of the project are as follows (unchanged from the annual report of May 2000). *Italicized text indicates updates.*

Task 1. To measure the response of spontaneous murine mammary carcinoma to combined radiation and antiangiogenic/antivascular therapies under variations of order and timing of the two modalities. (months 1 to 36)

- measure tumor response to 20 Gy (120 mice; months 1 to 9). *Completed task. Presented at Chemical Modifier/Tumor Physiology Meeting in Banff, Oct. 2000 (arsenic studies, see Appendix), Radiation Research Meeting in Puerto Rico, April 2001 (combretastatin studies, see Appendix), and being prepared for publication.*
  - radiotherapy before antiangiogenic/antivascular therapy  
Standard Therapy and Group A (40 mice; months 1 to 3)
  - antiangiogenic/antivascular therapy before radiation  
Group B (40 mice; months 3 to 6)
  - control experiments  
Group C (40 mice, months 7 to 9)
- measure tumor response to 10 or 30 Gy (100 mice; months 9 to 18). *This task is completed and is being prepared for publication. Presented at Chemical Modifier/Tumor Physiology Meeting in Banff, Oct. 2000 (arsenic studies, see Appendix), Radiation Research Meeting in Puerto Rico, April 2001 (combretastatin studies, see Appendix), and being prepared for publication.*
  - radiotherapy before antiangiogenic/antivascular therapy  
Standard Therapy and Group A (40 mice; months 9 to 12)
  - antiangiogenic/antivascular therapy before radiation  
Group B (40 mice; months 13 to 15)
  - control experiments  
Group C (20 mice, controls not repeated, months 16 to 18)

Task 2: To measure changes in tumor oxygen and blood flow following radiotherapy or antiangiogenic therapy (150 to 176 mice depending on statistics; months 1 to 36). *This task is on schedule overall. See details in each sub-task.*

- Imaging Group I: effect of antiangiogenic/antivascular therapy on oxygen  $^{19}\text{F}$  and electrode  $\text{pO}_2$  (48 to 64 mice; months 1 to 12). *This task is behind schedule with respect to  $^{19}\text{F}$  measurements and on target with respect to electrode  $\text{pO}_2$  measurements.*
- Imaging Group II: effect of radiation on tumor blood flow NMR and Iodo-AntiPyrine quantitative autoradiography (72 mice total split between two radiation doses; months 18 to 36). *This task is completed ahead of schedule with regards to autoradiography and remains on schedule with regards to NMR. Manuscript will be submitted in June 2001.*
- Imaging Group III: effect of antiangiogenic/antivascular therapy on tumor physiology in the same tumor over time (30 to 40 mice; months 13 to 18). *This task is on schedule.*

#### Key Research Accomplishments

- Blood flow changes are predictive of tumor response following drug therapy that is designed to target tumor vasculature.
- Radiation therapy sensitizes drug therapy with respect to tumor response in either a single dose regimen or a fractionated schema.
- Timing of radiation and drug therapy is critical to the magnitude of tumor response. If radiation follows drug administration, the effect of the therapies on tumor response is additive. If radiation proceeds drug delivery by 1 hour, the effect of the therapies on tumor response is more than additive.
- The magnitude of blood flow decrease 60 hours following the combined treatment of radiation and drug is consistent with and predicts increased tumor response.

#### Reportable Outcomes

1. Lew YS, Brown SL, Kolozsvary, A, Ryu S, Kim JH. Improved tumor response with combined radiation and vascular targeting agent, arsenic trioxide. In preparation 2001.
2. Brown SL, Wei L, Tavarekere TN, Kolozsvary A, Marshall M, Fenstermacher JD, Kim JH. Combretastatin reduced blood flow in a spontaneous murine tumor and enhances tumor response in combination with radiation. In preparation 2001.

3. Lew YS, Kolozsvary A, Brown SL, Song CW, Kim JH. Arsenic trioxide: a novel vascular targeting agent and radiosensitizer. Presented at the 11<sup>th</sup> Meeting of Chemical Modifiers of Cancer Treatment – Tumor Physiology, Banff, Alberta, Canada, October 4-8, 2000.
4. Brown SL, Wei L, Tavarekere TN, Marshall M, Kolozsvary A, Fenstermacher JD, Kim JH. Magnitude of radiosensitization by combretastatin depends on initial tumor blood flow. Presented at the 48th Annual Meeting of Radiation Research, San Juan, Puerto Rico, April 21-25, 2001.

### Conclusions

The results to date provide critical data on the use of drugs that have an effect on tumor blood flow particularly in combination with radiation therapy. The data indicates that antivascular therapy should follow radiation therapy by one hour for maximum effect. The combined effects of drug and radiation are more than additive with tumor response as the endpoint. Blood flow changes are predictive of tumor response both following drug alone and combined with radiation. There is considerable urgency to complete the non-invasive MRI physiological measurements since the techniques will be directly applicable to humans. The focus of year three is to complete task 2 as planned. The project is progressing on schedule.

### References

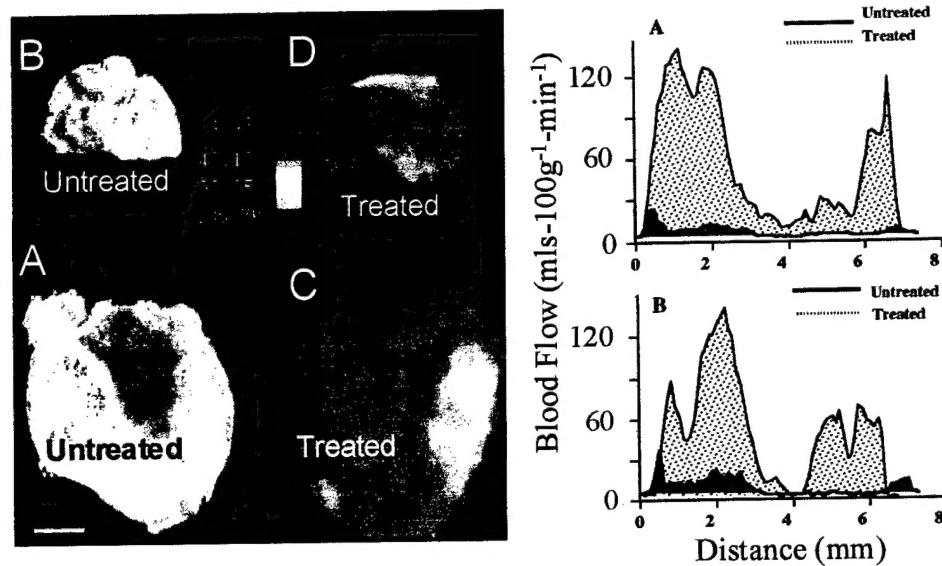
1. Lew YS, Brown SL, Griffin RJ, Song CW and Kim JH. Arsenic trioxide causes selective necrosis in solid murine tumors by vascular shutdown. *Cancer Research* 59: 6033-6037, 1999.
2. Lew YS, Kolozsvary A, Brown SL, Song CW, Kim JH. Arsenic trioxide: a novel vascular targeting agent and radiosensitizer. Presented at the 11<sup>th</sup> Meeting of Chemical Modifiers of Cancer Treatment – Tumor Physiology, Banff, Alberta, Canada, October 4-8, 2000. **see Appendix for Extended Abstract**
3. Brown SL, Wei L, Tavarekere TN, Marshall M, Kolozsvary A, Fenstermacher JD, Kim JH. Magnitude of radiosensitization by combretastatin depends on initial tumor blood flow. Presented at the 48th Annual Meeting of Radiation Research, San Juan, Puerto Rico, April 21-25, 2001. **see Appendix for Abstract**

Appendix

1. Figure 1: Quantitative autoradiography ( $^{14}\text{C}$ -Iodo-AntiPyrine) in murine spontaneous mammary carcinoma.
2. Figure 2: Tumor response to fractionated delivery of combretastatin and radiation.
3. Figure 3. Blood flow response to arsenic trioxide, radiation and combined therapies.
4. Lew YS, Kolozsvary A, Brown SL, Song CW, Kim JH. Arsenic trioxide: a novel vascular targeting agent and radiosensitizer. Presented at the 11<sup>th</sup> Meeting of Chemical Modifiers of Cancer Treatment – Tumor Physiology, Banff, Alberta, Canada, October 4-8, 2000.
5. Brown SL, Wei L, Tavarekere TN, Marshall M, Kolozsvary A, Fenstermacher JD, Kim JH. Magnitude of radiosensitization by combretastatin depends on initial tumor blood flow. Presented at the 48th Annual Meeting of Radiation Research, San Juan, Puerto Rico, April 21-25, 2001.

Figure 1. Quantitative autoradiography  
( $^{14}\text{C}$ -Iodo-AntiPyrine) in Murine Spontaneous Mammary Carcinoma

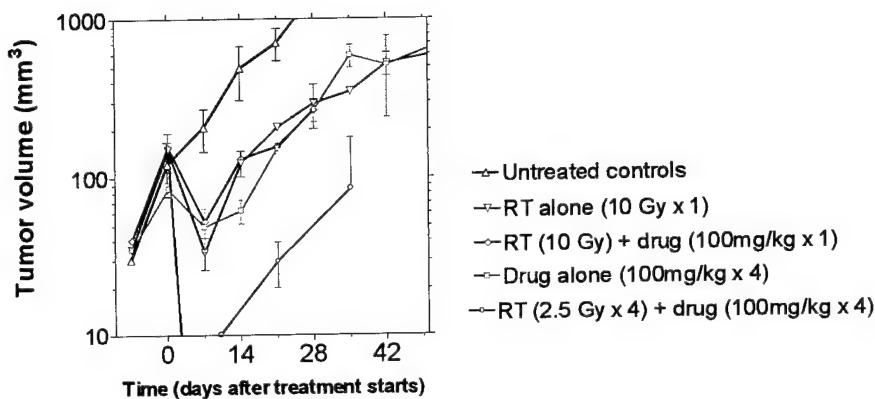
**Examples of Quantitative Autoradiography Results  
with Murine Spontaneous Mammary Carcinoma**



**METHODS AND MATERIALS**

Combretastatin-A4-P (provided by OxiGene Inc., Sweden) was administered i.p. at 100 mg/kg 1 hour before sampling of blood for flow measurements. Female Oncomice™ were purchased from Charles River Laboratories when they were approximately 42 days old. Spontaneous tumors grew when the mice reached puberty. Tumors had a volume doubling time of 6 days. Between 1 and 7 tumors grew per animal. Mice were used for blood flow studies when at least one tumor was 9mm in diameter. At the time of the experiment, mice were between 23 and 25g in weight. Tumor blood flow was measured using uptake of radiolabeled [ $^{14}\text{C}$ ]IAP (New England Nuclear). Mice were anesthetized using sodium pentobarbital at 60 mg/kg, 10 to 15 minutes prior to surgery. Left leg femoral artery and right leg femoral vein were catheterized with PE-10 tubing. Arterial blood pressure was monitored up to the point of blood flow measurement. At the appropriate time, blood flow was measured by infusing 5 $\mu\text{Ci}$  of [ $^{14}\text{C}$ ]IAP dissolved in 0.1 mls of buffered saline into the femoral vein for 20 s. During the 20 s period, free-flowing blood from the femoral artery was collected at 1 s intervals on pre-weighed filter paper. At the end of the 20 s, the mice were killed by decapitation. Tumors were removed as quickly as possible. In some experiments, brain and other tissues were also removed to assess blood flow in normal tissues. Tumors and tissues were frozen in 2-methylbutane cooled to 45°C with dry ice. Blood and tissue samples were counted using a scintillation well-counter. Radioactive counts per ml of blood as a function of time were determined from the ratio of radioactivity on filter paper divided by the difference between the weight of the blood soaked filter paper and the pre-weighed filter paper. Tumors and normal tissues were cryostat sectioned at 20 $\mu\text{m}$  thickness at intervals of 400 to 600 $\mu\text{m}$ , rapidly dried, mounted on cardboard and placed next to autoradiographic film along with appropriate standards for 10 days. The standards were used to construct a curve of nC as a function of optical density. Regions of darkened film were converted to nC from measurements of optical density using the standard curve. Blood flow rates to tumor and normal tissues were calculated from tissue counts, the equilibrium partition coefficient for IAP in the different tissues, and the arterial input function derived from the arterial blood counts. Autoradiographic images were digitized and converted to blood flow images pixel-by-pixel (AIS, Hamilton Ontario).

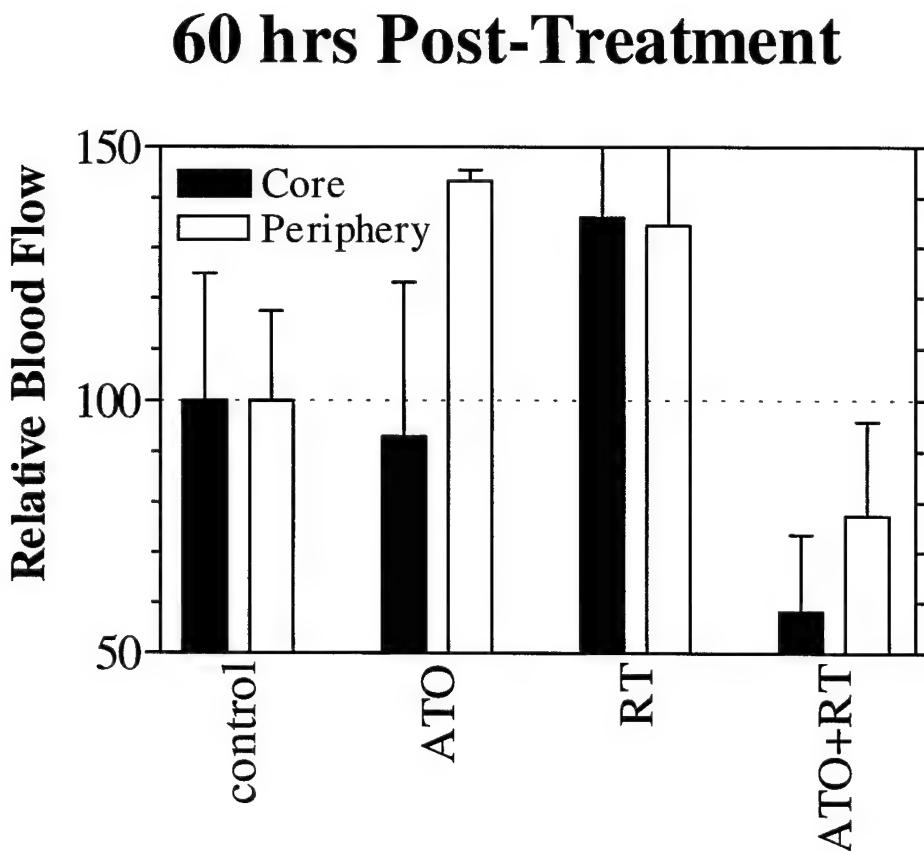
**Figure 2.**  
**Tumor Response to Fractionated Delivery of Combretastatin and Radiation**



Tumor response was enhanced with a relatively modest radiation and drug fractionation schedule. Daily fractions of radiation (2.5 Gy for 4 days) delivered one hour before combretastatin (100 mg/kg, i.p.) approximately doubled the growth delay observed with drug alone or radiation alone. Note that when drug was administered one hour following a single 10 Gy radiation dose, no additional growth delay was observed compared with 10 Gy alone.

More than additive effects were observed with a different vasoactive agent, arsenic trioxide, in a fractionated regimen. See Extended Abstract in Appendix, Lew YS, Kolozsvary A, Brown SL, Song CW, Kim JH. Arsenic trioxide: a novel vascular targeting agent and radiosensitizer. Presented at the 11th Meeting of Chemical Modifiers of Cancer Treatment – Tumor Physiology, Banff, Alberta, Canada, October 4-8, 2000.

**Figure 3.**  
**Blood flow response to arsenic trioxide, radiation and combined therapies.**



Rubidium ( $^{86}\text{Rb}$ ) uptake measurements of changes in blood perfusion were used to assess the antivascular effect of  $\text{As}_2\text{O}_3$  alone and in combination with radiation. In all experiments,  $5\mu\text{Ci}$  of  $^{86}\text{RbCl}$  in a 0.1ml volume were injected through a tail vein after anesthesia with 100mg/kg ketamine and 10mg/kg xylazine, and mice were sacrificed by cervical dislocation 60 seconds after injection. The periphery and core of tumors were excised and counted in a well-counter (Pharmacia LKB Nuclear, Inc., Gaithersburg, MD). The ratio of radioactive counts from the tissue of interest to the counts in the standard  $^{86}\text{Rb}$  solution equivalent to the total  $^{86}\text{Rb}$  activity injected multiplied by 100 gives the percentage of cardiac output to the tissue of interest. Relative blood perfusion values are presented.  $^{86}\text{Rb}$  uptake was measured before and at 24, and 60 hours after treatment.

At 24 hours after the combined administration of arsenic and radiotherapy, tumor blood flow was reduced. However, the magnitude of reduction with the combined drug plus radiation treatment was not statistically different from that obtained with arsenic alone (data not shown).

At 60 hours post-treatment, tumor blood flow remained low in tumors treated with the combined radiation and antivascular therapies, whereas tumors treated with drug alone had blood flows that had returned to pre-treatment values. The decrease in tumor blood flow was more pronounced in the tumor core than the periphery.

## ARSENIC TRIOXIDE: A NOVEL VASCULAR TARGETING AGENT AND RADIOSENSITIZER

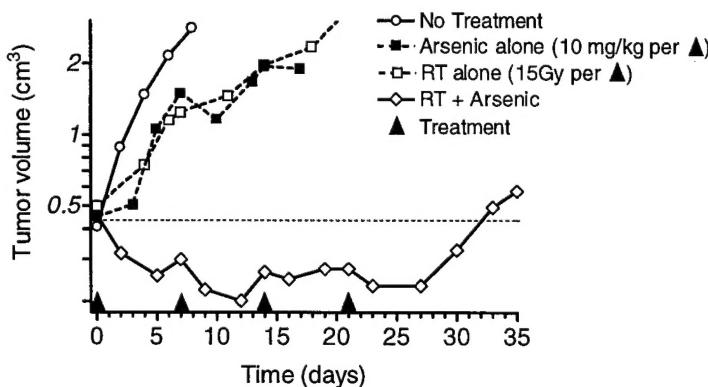
Young S. Lew<sup>1</sup>, Andrew Kolozsvary<sup>1</sup>, Stephen L. Brown<sup>1</sup>, Chang W. Song<sup>2</sup>, and Jae Ho Kim<sup>1</sup>

1. Henry Ford Health Science Center, 2799 W. Grand Blvd., Detroit, MI 48202.

2. University of Minnesota, 420 Delaware St, Minneapolis, MN 55455.

**Summary:** Arsenic trioxide has been shown to have an anti-tumor effect. Phase I and II clinical trials including both solid tumors and hematologic malignancies are ongoing. Our data indicates that arsenic trioxide causes vascular shut down in regions of ischemia, mostly in the central tumor area. The purpose of this study was to investigate the interaction of arsenic trioxide and radiation since they seem to exert their effects on different tumor compartments.

Figure 1. Radiation + ATO.  
The effect of combining radiation and arsenic trioxide on the growth of subcutaneous Meth-A leg tumors. This model was used because of its ease in assessing tumor response. The dose of radiation and drug was 12 Gy and 10mg/kg respectively, once a week four times. ATO was given 1 hour after radiation. Ten mice per group.



**Methods:** The effect on tumor growth of arsenic trioxide administered after radiation was assessed using methylcholanthrene-induced murine fibrosarcoma (Meth-A) grown in the right hind limbs of Balb/C mice. Tumor blood flow was assessed using <sup>99m</sup>TcO<sub>4</sub><sup>-</sup> clearance and <sup>86</sup>Rb uptake techniques. Tumor growth delay, defined as the additional time necessary for a tumor to grow to 1 cm in average diameter compared to untreated tumors, was determined for groups receiving radiation alone, drug alone, and combined therapies.

**Results:** A single administration of arsenic trioxide (10 mg/kg, i.p.) produced a preferential vascular shut down in the tumor tissue at 2 and 6 hours, leading to extensive necrosis of the tumor core. Normal skin, muscle, and kidney were relatively unaffected by arsenic trioxide. Based on the temporal effect of arsenic trioxide on tumor blood flow, radiation was delivered 1 hour before drug. Tumor growth delay was 2.5, 2.7, and 34.0 days for groups receiving radiation alone, drug alone, and both therapies, respectively. Similar synergistic interactions between drug and radiation were observed in the human tumor xenograft in nude mice.

**Conclusions:** The results have prompted further experiments, which are in progress to test two hypotheses. One hypothesis is that radiosensitization is mediated by tumor necrosis factor at the tissue level. The second separate but non-exclusive hypothesis is that the more than additive enhancement of radiation by arsenic trioxide results from improving tumor oxygenation in radioresistant hypoxic cells despite the reduction in blood flow.

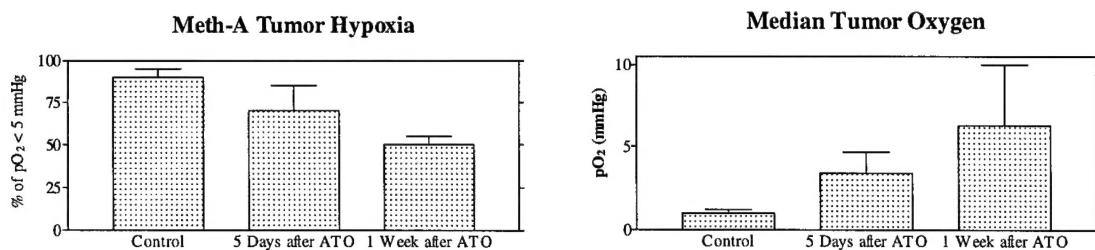


Figure 2. Oxygen polarographic measurements following ATO administration. Tumor oxygen was measured in over 100 locations in 8 to 10 tumors. The values presented represent average values and standard error of the means.

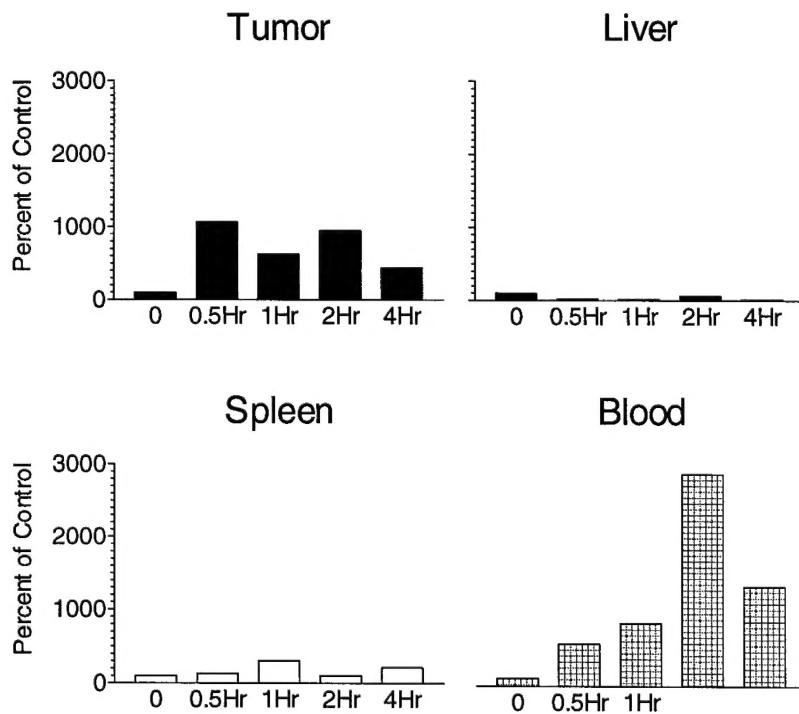


Figure 3. Bar graph of Tumor Necrosis Factor in different tumors after ATO. TNF $\alpha$  in intradermal tumor, liver, spleen and blood was measured following ATO, 10 mg/kg, i.p. Balb/C mice were anesthetized, blood collected by cardiac puncture, coagulated overnight at 4°C and centrifuged for 30min at 2000x g and 4°C. Tissue extracts were weighed and homogenized in ice cold PBS using a tissue homogenizer. The homogenates were centrifuged at 2000x g for 30 min at 4°C, and the supernatants removed and centrifuged at 14,000x g for 30min at 4°C. TNF $\alpha$  was measured by ELISA (Quantikine M, R&D Systems, Minneapolis, MN).

**Acknowledgements:** The authors thank Andrew J. Kolozsvary for preparing the manuscript.

plus ATO. The endpoints for evaluation were the median survival time and survival rates from the day of tumor implantation, and histologic changes of the brain with H&E, GFAP, and LFB/PAS stains. Pharmacokinetic studies were also performed. Results: Median survival times of untreated control animals and ATO (6-8 mg/kg) treatment were 19 and 18 days, respectively. Radiosurgery (25 Gy) increased the median survival to 39 days. Combined radiosurgery and ATO treatment almost doubled the median survival time to 73 days compared to radiosurgery alone group. Tumor cure rate was 50% at 120 days of follow-up in this combined treatment group. Conclusion: A significant radiosensitization and cure of the aggressive brain tumor was achieved by combined use of radiosurgery and ATO. This result provides a strong radiobiologic rationale for the combined use of radiotherapy and anti-vascular agent for treatment of the malignant brain tumors.

**(P26-346) Anti-vascular Strategies to Enhance Radiotherapy of Solid Tumors.** R.J. Griffin<sup>1</sup>, B.W. Williams<sup>1,\*</sup>, Y.S. Lew<sup>2,\*</sup>, K.L. Rood<sup>1,\*</sup> and C.W. Song<sup>1</sup> <sup>1</sup>University of Minnesota Medical School, Minneapolis, MN 55455 USA. <sup>2</sup>Henry Ford Health System, Detroit, MI 48202 USA.

We have investigated the potential of an anti-vascular drug, Arsenic Trioxide (ATO), to (1) assist radiation therapy by destroying hypoxic, radioresistant portions of tumors and (2) to enhance the activity of the hypoxic-cell-specific drug Tirapazamine using murine tumors. Previously, we discovered that 5-10 mg/kg ATO causes a semi-permanent vascular shutdown in solid tumors, evidently via oxidative stress to the tumor vasculature resulting in inflammation and vascular shutdown. Interestingly, we found the pO<sub>2</sub> in FSAll and MethA tumors, measured with an Eppendorf pO<sub>2</sub> histogram, was increased in the peripheral region of the tumor at 5-7 days after ATO treatment. Histological examination of the tumors from animals treated with ATO 5 days earlier revealed heightened interior necrosis as compared to control, especially in areas remote from blood vessels that were likely already hypoxic and acidic. However, a perimeter of tumor tissue remained viable and may have become better oxygenated through an increase in available oxygen due to the ATO induced cell death in the center of the tumor. The radiation-induced tumor growth delay caused by once weekly 12 Gy irradiation for 4 weeks was substantially increased in FSAll and MethA tumors when 8-10 mg/kg ATO was injected 2 h after each irradiation. We also tested if ATO-induced vascular shutdown could enhance the anti-tumor effect of Tirapazamine. We found that ATO, given 15 min after Tirapazamine, enhanced the effect of Tirapazamine on tumor growth delay by 1.2-1.4 fold, indicating that ATO may create an optimal environment for Tirapazamine to exert hypoxic cell cytotoxicity. Supported by grant CA13353 from the NIH.

**(P26-347) Magnitude of Radiosensitization by Combtastatin Depends on Initial Tumor Blood Flow.** S.L. Brown<sup>1</sup>, L. Wei<sup>1,\*</sup>, T.N. Nagarajan<sup>1,\*</sup>, M. Marshall<sup>1,\*</sup>, A.J. Kolozsvary<sup>1,\*</sup>, J.D. Fenstermacher<sup>1,\*</sup> and J.H. Kim<sup>1</sup> <sup>1</sup>Henry Ford Health System.

Combtastatin-A4-P (provided by OxiGene Inc., Sweden), an aromatic hydrocarbon derived from the root of an African Willow Tree, causes a preferential reduction in tumor blood flow compared with normal tissue blood flows. Furthermore, Combtastatin-A4-P delivered after radiation enhances radiation induced tumor-growth-delay (TGD). In this study, we extended the TGD studies to a mouse spontaneous tumor model. We tested the hypothesis that the magnitude of radiosensitization by combretastatin-A4-P correlates with initial tumor blood flow. Studies were performed using DuPont mice, a transgenic mouse that produces mammary carcinoma at puberty (Charles River Laboratories, Wilmington, MA). Tumors grow at three sites: cervical (neck), lateral thorax (fossa axillaris, front limb pits), and inguinal (groin). Combtastatin-A4-P was administered in 0.2 mls at 100mg/kg. Radiation dose was 10 Gy, delivered using a cesium irradiator. Drug delivery followed radiation by 1 hour. At least 6 tumors and 4 mice were included in each experimental group: untreated control, radiation alone, drug alone, radiation plus drug. The endpoint of the studies was TGD. Blood flow was measured using quantitative autoradiography to assess tumor blood flow in untreated mice (3 mice, 6 tumors) or tumor blood flow one hour after 100 mg/kg Combre-

tastatin-A4-P delivery (4 mice, 11 tumors). The effect of drug plus radiation on tumor growth delay was at least additive compared with the effect of drug alone or radiation alone on tumor growth delay. There was no obvious dependency of TGD or blood flow on tumor size. Response of tumors to drug alone as well as combined drug plus radiation was greatest for inguinal tumors and least for lateral thorax tumors. Consistent with the hypothesis, pre-treatment blood flow was greatest in the inguinal tumors and lowest in the lateral thorax tumors.

**(P26-348) Differential effects of NOS inhibitors on tumour blood flow: role in vascular targeting.** G.M. Tozer<sup>1,\*</sup>, V.E. Prise<sup>1,\*</sup> and J. Wilson<sup>1,\*</sup> <sup>1</sup>Gray Laboratory Cancer research Trust, Nortwood, Middx., HA6 2JR, UK..

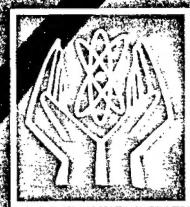
Combretastatin A4-P (CA-4-P) is a tubulin-binding agent in Phase I clinical trial as a tumour vascular targeting agent. The current study aimed to investigate the role of endogenous tumour nitric oxide (NO) levels in the response to CA-4-P. Blood flow was measured, in BD9 rats bearing sc P22 tumours, using the uptake of <sup>125</sup>I-labelled iodo-antipyrine. L-NNA (N<sup>ω</sup>-nitro-L-arginine) and its pro-drug L-NAME (the methyl ester of L-NNA), which inhibit all isoforms of NO synthase (NOS), selectively reduced tumour blood flow (to approximately 50% and 80% of control respectively, at 15-30 mins after i.v. treatment). The relatively selective inducible NOS (iNOS) inhibitors, 1400W, aminoguanidine and aminoethyl-isothiourea had no effects on normal or tumour tissue blood flow. Chronic administration of L-NAME in the drinking water (1 mg/ml for 24h) reduced tumour blood flow to 24% of control and its combination with CA-4-P treatment, given at the end of the 24h period, produced a more than additive effect on tumour blood flow reduction. However, acute administration of L-NAME or L-NNA around the time of CA-4-P treatment produced only an additive effect. In normal tissues, combined treatments produced very similar blood flow effects to treatment with CA-4-P alone. The inactive D-isomer, D-NAME, had very little effect. In conclusion, constitutively produced NOS, rather than iNOS, has an important role in the maintenance of blood flow in the P22 tumour. A vascular effect of chronic NOS inhibition, rather than acute tumour blood flow reduction at the time of CA-4-P administration, sensitises the tumour vasculature to the damaging effects of CA-4-P. Elucidating the precise mechanism of action of this effect has implications for optimising the use of vascular targeting agents. *This work was funded by the Cancer Research Campaign and the National Lottery Charities Board of the UK. CA-4-P was supplied by OxiGene Inc.*

**(P26-349) Motexafin Gadolinium: A tumor selective radiation enhancer for treatment of brain metastases.** R.A. Miller<sup>1,\*</sup>, M.F. Renschler<sup>1,\*</sup>, S.C. Phan<sup>1,\*</sup> and M.P. Mehta<sup>2,\*</sup> <sup>1</sup>Pharmacyclics, Inc.. <sup>2</sup>University of Wisconsin.

Motexafin gadolinium (MGd) is a tumor selective drug that enhances radiation responsiveness by futile redox cycling and is now under evaluation in a randomized phase III trial in patients with brain metastases receiving whole brain radiation. 25 patients were treated in a lead-in phase of the trial and are reported here. Patients received 5mg/kg of MGd prior to each of 10 fractions of radiation of 3Gy. Clinical evaluations included toxicity, radiologic response, neurologic progression and survival. Patients had advanced disease: 56% uncontrolled primary, 84% extracranial metastases, median 5 lesions per patient. Overall, the drug was well tolerated with 94% of planned drug doses delivered; no grade 3/4 toxicity. MGd selectively accumulated in tumors. The radiologic response rate was 68% by MRI with median tumor volume reduction of 81%. The median survival was 5.0 months with a freedom from neurologic progression of 77% at 1 year. MGd appears to improve local control in patients with brain metastases. Neurologic progression, including neurocognitive testing, is an important clinical parameter in the evaluation of patients with brain metastases.

Dr. Richard Miller is an employee and shareholder of Pharmacyclics, the commercial developer of motexafin gadolinium.

PROGRAM & ABSTRACTS



Fourteenth Annual Meeting of the  
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